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AFFINITY FIBRE — A NEW SUPPORT FOR RAPID ENZYME PURIFICATION BY HIGH-PERFORMANCE LIQUID AFFINITY CHROMATOGRAPHY

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SUMMARY

A new type of support for chromatographic use has been developed. Non-porous quartz fibre, with a mean diameter of 0.5 μm, was silylated with mercaptopropyltrimethoxysilane. Tresyl chloride-activated dextran was covalently coupled to the SH groups on the fibre. Remaining active tresyl groups on the dextran were then coupled with an NAD derivative.

The affinity fibre contained 0.3 μmol NAD derivative per g and was able to bind 15 mg of lactate dehydrogenase per g of fibre.

The affinity fibre was used for large-scale purification of ox heart lactate dehydrogenase and its performance was compared with other commonly used chromatographic matrices. The operational capacity was found to be 1.0 g of pure lactate dehydrogenase per hour per 100 g opf fibre material (adsorption followed by salt elution). The affinity fibre was found to be particularly suitable for very rapid processing of large volumes of dilute enzyme solutions.

INTRODUCTION

Affinity chromatography is by now a well established technique for protein purification (see refs. 1 and 2 and papers cited therein). The prime characteristic of the technique is its often very pronounced selectivity for one or a few compounds, usually proteins, a fact that has made it eminently suitable for preparative purposes. The recent application of high-performance liquid chromatography (HPLC) technology to affinity chromatography has promoted the analytical applicability of the latter[3–8], as well as improved its preparative performance[4,9].

The traditional support materials for affinity chromatography are soft gels such as spherical agarose beads (Sepharose). Recent developments regarding these soft gel supports have made them more pressure-resistant, which is necessary if high flow-rates are desired. High-performance liquid affinity chromatography systems are usually based on highly pressure resistant, small-sized silica particles. However, such small particles are sensitive to contamination by the applied sample.

In the present paper we describe a new affinity material —an affinity fibre— with suitable properties for both preparative and analytical affinity chromatography. The affinity fibre is prepared from non-porous quartz fibres with a very small di-

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ameter (0.5 μm). Due to its non-porous nature, the affinity fibre equilibrates very rapidly with the mobile phase. As a consequence, a very fast flow is possible through an affinity fibre column while resolution can be maintained.

MATERIALS AND METHODS

Quartz fibre (Q 106), was purchased from Johns-Manville (Denver, CO, U.S.A.) and cut in a homogenizer to an average length of 50 μm. Sepharose CL-6B and dextran T 500 were obtained from Pharmacia (Uppsala, Sweden), Fractosil 500 and LiChrospher Si 1000 from E. Merck (Darmstadt, F.R.G.), mercaptopropyltrimethoxysilane and γ-glycidoxypropyltrimethoxysilane (Z-6040) from Dow Corning (Seneffe, Belgium) and tresyl chloride (2,2,2-trifluoroethanesulphonyl chloride) from Fluka (Buchs, Switzerland). The NAD analogue, N^6-[N-(2-aminoethyl)carbamoylmethyl]-NAD, was synthesized according to a method described in the literature[10].

Preparation of diol silica (Fractosil 500, LiChrospher Si 1000)

The procedure used to epoxysilylate Fractosil 500 and LiChrospher Si 1000 was a modification of a previously published method[11]. The silica (2 g) was suspended in 200 ml of toluene (dried over sodium) and 1 ml of γ-glycidoxypropyltrimethoxysilane was added (2 ml in the case of Fractosil 500). To catalyse the reaction 0.1 ml of triethylamine was added. The slurry was allowed to reflux for 8 h with continuous stirring. The epoxy group concentration of the silica was determined by reaction with sodium thiosulphate and titration of released OH^-[12]. Fractosil 500 had 200 μmol epoxy groups/g and LiChrospher Si 1000 had 90 μmol/g. The epoxy-silica (Fractosil and LiChrospher) was converted to diol-silica by acid hydrolysis. The gels were suspended in 1 l of water acidified to pH 2 with sulfuric acid and stirred at 70°C for 2 h.

Preparation of mercaptopropyl-fibre (Fig. 1)

The fibres (90 g) were packed in a stainless-steel column, which was subsequently washed with five column volumes each of acetone, dry acetone and dry toluene. The temperature of the column was held constant at 90°C and a mixture of 400 ml dry toluene, 1 ml mercaptopropyltrimethoxysilane and 0.1 ml triethylamine recirculated through the fibre column for 8 h. Then the column was washed with dry toluene, acetone, ethanol and water.

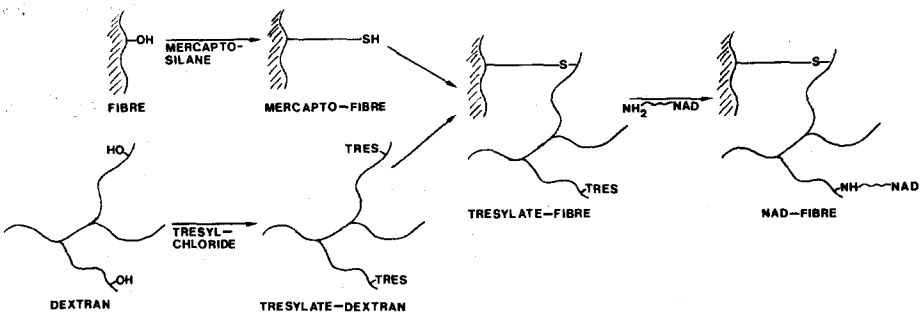

Fig. 1. Fibre derivatization procedure.

Preparation of NAD-bonded Fractosil 500 and LiChrospher Si 1000

The diol-silica (see above) was activated with tresyl chloride, according to a published procedure[13]. For 2 g of dry diol-silica, 5 ml dry acetone, 0.2 ml pyridine and 0.1 ml tresyl chloride were used and for 2 g of Fractosil, 5 ml acetone, 0.4 ml pyridine and 0.2 ml tresyl chloride. The slurry was thoroughly mixed and was allowed to react on a rocking table for 1 h. The activated silica was washed with 100 ml of each of the following washing mixtures: acetone, acetone–5 m*M* hydrochloric acid (70:30, 50:50, 30:70), and 5 m*M* hydrochloric acid. The silica gels were then washed with a coupling buffer of 0.1 *M* sodium bicarbonate, pH 8. The silica gels were then suspended in 4 ml of the same buffer containing 20 mg of the NAD derivative N^6-[N-(2-aminoethyl)carbamoylmethyl]-NAD, (40 mg in the case of Fractosil 500). The slurry was placed in a test-tube with a screw cap, and was allowed to react at room temperature for 20 h. Unreacted tresyl groups were then blocked with 0.2 *M* Tris–HCl, pH 8, overnight. The amount of NAD derivative coupled to LiChrospher Si 1000 was 7 μmol, as determined by UV measurements.

Preparation of NAD-bonded fibre (Fig. 1)

Dextran T 500 (18 g) was dried in an oven (100°C) for 4 h and was then dissolved in 270 ml dry dimethyl sulphoxide (dried with 4 A molecular sieve). Dry pyridine, 4 ml, was added followed by 2 ml tresyl chloride, while the solution was vigorously stirred. The reaction was carried out under dry conditions and was allowed to proceed for 6 h. Non-reacted tresyl chloride was destroyed and a suitable pH for the subsequent coupling reaction was achieved by adding 90 ml 0.1 *M* sodium bicarbonate (pH 8). The solution of activated dextran was recirculated through a stainless-steel column, packed with 90 g of mercaptopropyl–silylated fibre, with a flow-rate of 10 ml/min (overnight).

The dextran fibre thus obtained was washed *in situ* with 3 l 0.1 *M* sodium bicarbonate (pH 8.0). The NAD derivative N^6-[N-(2-aminoethyl)carbamoylmethyl]-NAD (200 mg) was dissolved in 100 ml 0.1 *M* sodium bicarbonate (pH 8.0), and recirculated through the column overnight at room temperature. Non-reacted tresyl groups were then blocked by circulating 1 l 0.2 *M* Tris–HCl buffer (pH 8.0) overnight. The amount of NAD coupled was determined by hydrolysing the dextran-NAD derivative at low pH (1 *M* hydrochloric acid at 80°C) and measuring the amount of released nucleotide material by its absorbance at 266 nm. A value of 0.33 μmol NAD analogue/g fibre was obtained.

Preparation of NAD-bonded Sepharose

Sepharose CL-6B (10 g wet weight) was activated with tresyl chloride according to an earlier published method[13]. The gel was washed with 300 ml distilled water, and 100 ml of each of the following washing solutions: acetone–distilled water (30:70, 70:30, 85:15) and then 2 × 100 ml acetone and 3 × 100 ml dry acetone. The moist gel was then slurried in 4 ml acetone. To the slurry was added, 0.15 ml dry pyridine and 0.1 ml tresyl chloride and the reaction was allowed to proceed for 2 h. The following washing solutions were then used: 100 ml acetone, 100 ml 5 m*M* hydrochloric acid–acetone (30:70, 50:50, 70:30), 200 ml 5 m*M* hydrochloric acid. Immediately before coupling, the gel was washed with coupling buffer, 0.1 *M* sodium bicarbonate (pH 8.0) and then suspended in 5 ml of the same buffer, containing 48 mg of

the NAD analogue N^6-[N-(2-aminoethyl)carbamoylmethyl]-NAD. The coupling was performed overnight at room temperature. Non-reacted tresyl groups were blocked with 0.2 *M* Tris–HCl. The amount of NAD coupled was determined by UV measurements to be 2.5 μmol NAD derivative/g Sepharose (wet weight).

Enzyme assay

The lactate dehydrogenase enzyme activity was assayed by following the oxidation of NADH by pyruvate at 340 nm and 22°C. The enzyme sample, 0.10 ml, was added to an end volume of 3.1 ml, which contained 0.21 m*M* NADH and 1.0 m*M* sodium pyruvate in 0.05 *M* sodium phosphate buffer (pH 7.0).

Protein determination

The protein concentration was determined according to Bradford[14]. Ox heart lactate dehydrogenase (Sigma grade III), was used as the protein standard.

Electrophoresis

The purity of the lactate dehydrogenase preparations was determined by agarose gel electrophoresis[15]. Separated proteins were stained with Coomassie brilliant blue R-250 and lactate dehydrogenase was also detected using a specific activity staining technique[16].

Packing of columns

Columns for analytical or micropreparative work were standard HPLC columns (50 × 5 mm) with compression fittings. Adsorbents based on LiChrospher Si 1000 and fibres were slurry-packed[17]. Adsorbents based on Fractosil 500 were packed by the tap-and-fill method[18]. Preparative fibre columns (*e.g.* 100 mm diameter, 10 mm height) were conveniently packed by filling the column and an extension at the top of the column with a fibre suspension and then applying a vacuum to the bottom of the column.

Purification of lactate dehydrogenase on an NAD-fibre column (typical procedure)

Ox heart (4000 g) was cut in small pieces, mixed with 8 l cold sodium phosphate buffer, pH 7.0, and homogenized in a kitchen type mixer for 5 min at 0–4°C. The homogenate was centrifuged at 20 000 *g* for 15 min. The supernatant was subsequently fractionated by ammonium sulphate precipitation and the fraction obtained between 30% and 65% saturation isolated and dissolved in 4 l sodium phosphate buffer, pH 7.0 (crude extract). The extract contained per ml 0.67 mg lactate dehydrogenase (activity assay) and 25 mg protein.

Potassium oxalate was added to the enzyme extract to a concentration of 0.025 *M* (to promote affinity binding). The extract was applied to an NAD-fibre column at room temperature. The column contained 90 g of fibre material and had a diameter of 100 mm and a length of 50 mm. The flow-rate was 100 ml/min giving a pressure of less than 5 bar.

After application of 3 l extract, the column was saturated with enzyme. The column was washed with 1.0 l of 0.05 *M* phosphate buffer (pH 7.0), containing 0.025 *M* potassium oxalate. The enzyme was finally eluted with 1 *M* sodium chloride at a flow-rate of 20 ml/min.

RESULTS AND DISCUSSION

A limiting factor when chromatographing large molecules such as proteins, is the slow diffusion within the pores of ordinary chromatographic support materials. In contrast, chromatography based on non-porous particles should be very efficient since diffusion in the pores is not a limiting factor. As a consequence very high flow-rates should be possible without deterioration or the separation efficiency. Non-porous particles must, however, be very small (sub-micrometer size) to offer a sufficient binding surface, at least for preparative applications. Further, a serious practical problem with such small particles would be the very high pressure drop generated over a column even at modest flow-rates. Such packing materials would also be very sensitive to contamination with solid particulates.

To exploit the inherent advantages of non-porous, small particles and at the same time avoid their problems, we have chosen small-diameter non-porous quartz fibres as the base material for a new chromatographic medium—the affinity fibre. The affinity fibre material is chromatographically efficient due to its surface-bound ligands. A fibre bed may process complex proteinous solutions and gives only a moderate pressure drop, even at high flow-rates. This may be partially explained by the structure of the chromatographic bed. A fibre bed will not be as densely packed as a bed with spherical or irregularly shaped particles of the same diameter (the haystack principle).

Design of the affinity fibre

The fibres chosen in this study have a mean diameter of 0.5 μm and a surface area of about 2 m^2/g. Initial experiments with this fibre involved direct attachment (epoxy silylation) of an NAD analogue to the fibre. The enzyme binding capacity of this material proved, however, to be disappointingly low, considerably lower than could be expected from geometrical considerations. A coating of high-molecular-weight dextran (MW = 500 000) proved to be a most satisfactory remedy, increasing the binding capacity for lactate dehydrogenase by a factor of over 100. Apparently, the dextran coating provided the affinity ligand with a flexible spacer which improved its interaction with the high-molecular-weight enzyme. The coating, although of molecular dimensions according to elementary analysis, probably forms a network around the fibre, in which several layers of enzyme molecules may be harboured. Saturation of such spacer-equipped NAD fibres with lactate dehydrogenase gave a binding capacity of about 15 mg of enzyme/g fibre. This is approximately twice the value expected from a close-packed pattern of enzymes on naked fibres.

The present fibre derivatization procedures are schematically illustrated in Fig. 1. The quartz fibre is initially silanized with mercaptopropyl silane to give the fibre a chemically reactive coating (nucleophilic SH groups). Dextran, activated with an excess of tresyl chloride, is then attached to the fibres via thioether linkages. The excess of tresylate groups on the dextran allows for the direct subsequent attachment of the NAD analogue, N^6-[N-(2-aminoethyl)carbamoylmethyl]-NAD via its terminal amino group.

An attractive practical feature of the fibre derivatization scheme is that the whole reaction sequence may be carried out as a "one-pot" procedure, *i.e.* the native fibre is packed in a large column and all the necessary reagents are consecutively pumped through the column until the derivatization is complete.

Tresyl chloride activation is a very powerful and, at the same time, gentle way of immobilizing proteins and affinity ligands[13]. The activation step requires the absence of water, a condition which is easily met when activating insoluble supports. In the present case where a soluble polymer, dextran, was used, dry dimethyl sulfoxide proved to be a suitable solvent and reaction medium for the activation procedure.

Small particles at high flow-rates

Three different well known matrices were compared with the new fibre material with respect to performance at high flow-rates:

(i) Sepharose Cl-6B, a gel which was successfully used in large-scale protein purification[19], and what consists of spherical agarose beads with a mean diameter of about 100 μm and very large pores;

(ii) LiChrospher Si 1000, an HPLC packing material consisting of large-pore, spherical silica particles with a mean diameter of 10 μm;

(iii) Fractosil 500, a preparative-grade silica consisting of irregularly shaped particles with a mean diameter of 50 μm and with large pores.

All matrices were substituted with the NAD analogue, N^6-[N-(2-aminoethyl) carbamoylmethyl]-NAD.

The performance of the different affinity materials was tested by pumping a solution of lactate dehydrogenase continuously through columns packed with the affinity materials (HPLC steel columns, 50 × 5 mm) and monitoring the effluent at 280 nm to detect the breakthrough of the enzyme. The enzyme solution contained an excess of oxalate which promoted a strong and rapid binding of lactate dehydrogenase to the affinity support as a ternary complex (enzyme–NAD–oxalate)[20]. Thus, any degeneration of the performance at increased flow-rate was indicative of a limiting diffusion rate of the affinity material tested.

Fig. 2 shows the results when the affinity packing materials were tested in the flow range 0–8 ml/min (0–40 cm/min linear velocity). The large particles of Sepharose

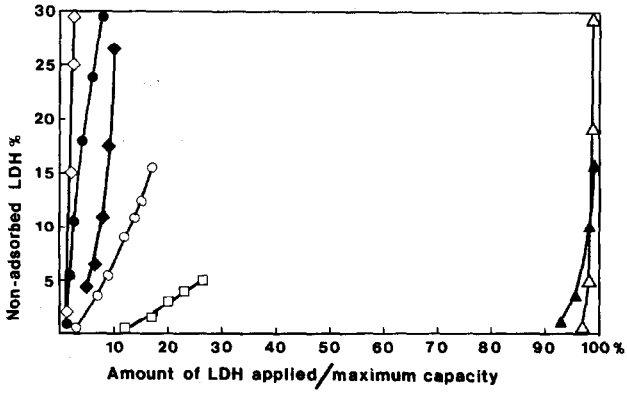

Fig. 2. Comparison of different NAD matrices by frontal analysis. Pure lactate dehydrogenase dissolved in 0.05 *M* phosphate buffer, pH 7.0, containing 0.025 *M* potassium oxalate, was pumped through a 50 × 5 mm column and the effluent monitored at 280 nm. The column contained NAD-Fractosil, NAD-Sepharose or NAD-fibre with maximum enzyme binding capacity of respectively 34, 48 and 5.4 mg/ml. NAD-Sepharose: 0.5 ml/min (□), 1.1 ml/min (○), 3.1 ml/min (●); NAD-Fractosil 500: 1.0 ml/min (◆), 3.0 ml/min (◇); NAD-LiChrospher Si 1000: 3.1 ml/min (▲); NAD-fibre: 1.1–8.1 ml/min (△).

Cl-6B only permitted slow flow-rates for efficient adsorption. Otherwise the diffusion of the enzyme in the pores to the binding sites is not fast enough to match the incoming flow of enzyme, with the result that a proportion of the enzyme will pass the column unbound. NAD-Fractosil 500 behaved in a similar way. Fractosil 500 would be expected to be more efficient than Sepharose in binding lactate dehydrogenase at increasing flow-rates, because of its particle size (Fractosil $\approx$ 50 μm and Sepharose $\approx$ 100 μm). The reason for the opposite result, is probably the smaller pore size of Fractosil 500.

NAD-LiChrospher Si 1000 showed, as expected from its small particle diameter ($\approx$ 10 μm), a very efficient binding of lactate dehydrogenase. At 3.1 ml/min the LiChrospher-based NAD gel was close to saturation before breakthrough of the enzyme. However, the gel was very unstable at higher flow-rates, and at 6.1 ml/min the gel collapsed before it was half saturated with lactate dehydrogenase.

Finally, Fig. 2 illustrates the superior performance of the affinity fibre. Close to 100% of the column capacity was utilized before enzyme breakthrough, at all the investigated flow-rates (1.1–8.1 ml/min). Furthermore, and equally important, no stability problems whatsoever were observed at any flow-rate.

Pressure versus flow-rate

Fig. 3 shows the relationship between pressure and flow-rate for fibre, Sepharose Cl-6B and Fractosil 500. Underivatized gels were packed in HPLC stainless steel columns (50 × 5 mm). The liquid that was pumped through the column contained 2 mg/ml of bovine serum albumin (model protein) dissolved in water. The Sepharose column collapsed at a flow-rate of 3–4 ml/min, but the gel could be used again, after repacking. As expected, the large Fractosil 500 particles gave a very low pressure drop, while the fibre gave a moderate pressure drop.

Purification of lactate dehydrogenase from bovine heart using NAD-fibre

Apartially purified lactate dehydrogenase preparation was used to evaluate the capacity of the affinity fibre in large-scale purification schemes. The enzyme prepa-

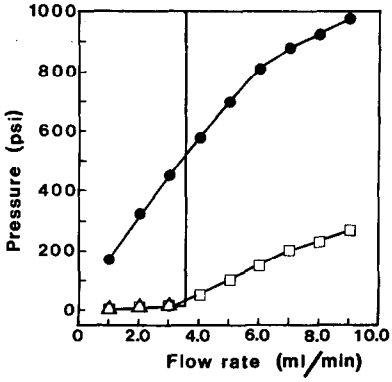

Fig. 3. Pressure as a function of flow-rate. Bovine serum albumin (2 mg/ml) in 0.05 *M* phosphate buffer pH 7.0 was pumped through a HPLC column (50 × 5 mm) and the pressure registered as function of the flow. The support materials tested were: Sepharose (△), Fractosil 500 (□) and silica fiber (●). The Sepharose column collapsed when going from 3 to 4 ml/min.

ration was obtained from an ammonium sulphate-precipitated crude extract and contained 25 mg protein/ml. Fig. 4 shows one purification cycle on a 0.4-l column containing 90 g of fibre. The complete cycle lasted less than 1.5 h and during this time 1.37 g of enzyme were isolated. The purity of the isolated enzyme was checked by electrophoresis and by activity measurements, and was estimated to be greater than 98%.

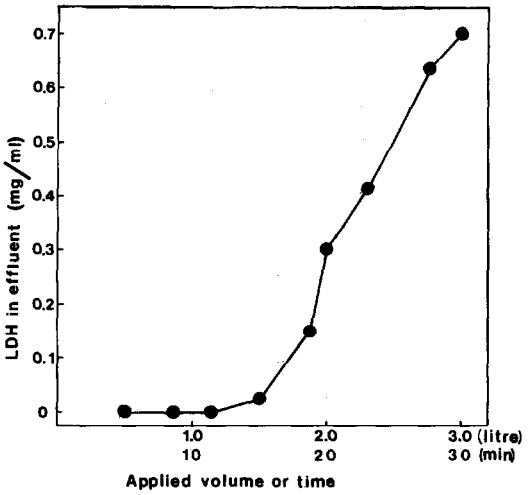

Fig. 4. Adsorption of lactate dehydrogenase from a crude extract on a preparative NAD-fibre column. The crude extract contained 25 mg protein/ml and 0.67 mg enzyme/ml (see Materials and methods section). The extract was pumped onto a short preparative column (50 × 100 mm I.D.) containing 90 g of NAD-fibre. The column effluent was sampled and analysed with respect to lactate dehydrogenase activity.

The results convincingly demonstrate the potential of affinity fibres for large scale purification purposes, for instance in biotechnological down-stream processing schemes. The capacity shown here is about 10 g of purified enzyme per h and kg of adsorbent, a capacity by far exceeding the capacities of earlier reported similar systems. Furthermore, the high capacity should be considered together with the purity achieved (>98%), the purification factor (about 35 ×), and the enzyme recovery (>90%).

The remarkable overall performance of the affinity fibre system is admittedly not only due to the properties of the fibre material itself but also due to the favorable binding properties of the biomolecules involved. However, crucial factors for the rapid preparative isolation of the enzyme are the rapid equilibration with the mobile phase and the stability at high flow-rates. The fact that the fibre column has a comparatively low volumetric capacity, about 4 mg of lactate dehydrogenase per ml of bed volume compared with more than 25 mg/ml for Sepharose and Fractosil 500, is amply compensated by the very high recycling rate possible with the fibre material. Also, utilizing the Sepharose and Fractosil adsorbents close to their maximum bind-

ing capacities is probably not without complications. Non-specific protein-protein interactions might be pronounced during the loading phase and partial precipitation might occur during the elution phase unless both phases are carried out carefully.

Fast flow—large volumes

The processing of very large volumes of dilute enzyme protein solutions should be particularly suitable for fibre adsorbents. The low protein content would give a low viscosity and the possibility of very high flow-rates. Fig. 5 gives an illustration. A short preparative column (100 mm diameter, 10 mm height), with a binding capacity of 280 mg of enzyme, was used. A solution containing 5.8 mg lactate dehydrogenase/l, was pumped through the column at a rate of 0.9 l/min (maximum rate for the pump used) until the column was saturated with the enzyme. This was achieved after 70 l. A more adequate end-point for effective usage of the column would be to stop at 20% leakage, that is after 40 l when 80% of the column capacity would be used.

The break-through curve in Fig. 5 is not as steep as the one shown for the same fibre material in Fig. 2. The reason is probably that the large preparative column (Fig. 5) had a lower packing density (0.24 g/ml instead of 0.36 g/ml) which might lead to bed irregularities and an uneven flow profile through the bed.

Stability of the binding capacity

The fibre bed showed good stability and reproducibility (Fig. 6), when pure lactate dehydrogenase was repeatedly injected and eluted on a micropreparative column (50 × 5 mm), that was packed with NAD-fibre to a bed height of 5 mm. The rest of the column was filled with glass beads (75–100 μm), de-activated with glycerolpropylsilane to eliminate any unspecific binding. The reason for the small bed height was to keep the column capacity of lactate dehydrogenase low during the 200 cycles the stability test was scheduled for. Loading of the column with lactate de-

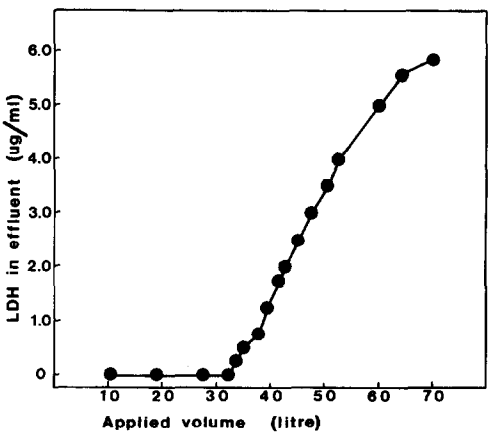

Fig. 5. Frontal analysis of lactate dehydrogenase in a fast-flow binding experiment. A dilute enzyme solution (5.8 mg/l) in 0.05 *M* sodium phosphate buffer, pH 7.0, containing 0.025 *M* potassium oxalate was applied to an NAD-fibre column at a flow-rate of 0.91 l/min (maximum rate for the pump used). Column dimensions: 10 × 100 mm I.D. The column effluent was analysed with respect to lactate dehydrogenase activity.

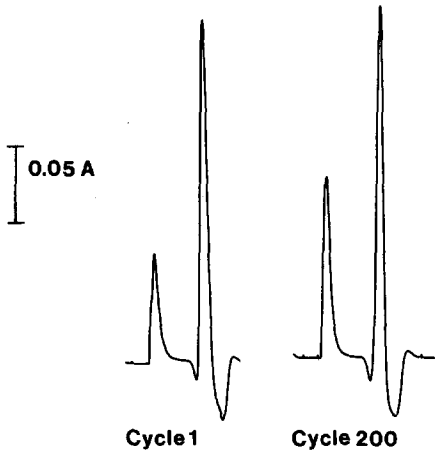

Fig. 6. Reproducibility of binding capacity (pure enzyme). A solution of pure lactate dehydrogenase (0.63 mg/ml) in 0.05 *M* sodium phosphate buffer, pH 7.0, containing 0.025 *M* potassium oxalate was injected on a 5 × 5 mm NAD-fibre column. The injected volume (about 1.2 ml) saturated the column (the first peak in both diagrams represents the excess of enzyme). After 4 min the adsorbed enzyme was eluted with 1 *M* sodium chloride (second peak). Flow-rate, 1.0 ml/min. Total cycle time, 12 min.

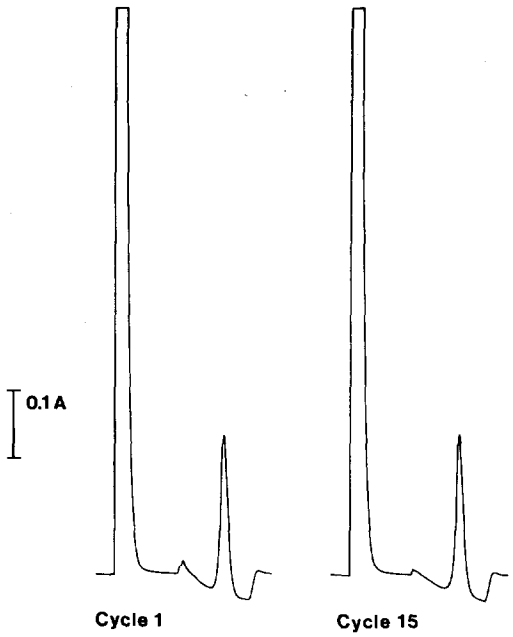

Fig. 7. Reproducibility of binding capacity (crude enzyme extract). An enzyme extract (0.5 mg enzyme/ml) in 0.05 *M* phosphate buffer, pH 7.0, containing 0.025 *M* potassium oxalate was injected on a 5 × 5 mm NAD-fibre column. The injected volume (about 1.2 ml) saturated the column (the first peak in both diagrams represents the excess of lactate dehydrogenase and other non-binding proteins). After 12 min the adsorbed enzyme was eluted with 1 *M* sodium chloride. Flow-rate, 1.0 ml/min. Total cycle time, 24 min.

hydrogenase occurred in the presence of 25 m*M* potassium oxalate in 0.05 *M* sodium phosphate buffer (pH 7.0) and elution of the enzyme was achieved using 1 *M* sodium chloride in 0.05*M* phosphate buffer as eluent. Between the cycles the column was equilibrated with 25 m*M* potassium oxalate in phosphate buffer. Fig. 6 depicts cycles 1 and 200. Peak number one in both cycles, is lactate dehydrogenase that did not bind, because of over-saturation of the column. The reason that these peaks are of different heights, is due to a slight inconsistency when supplying lactate dehydrogenase with a peristaltic pump. Peak number two, which belongs to the eluted lactate dehydrogenase, shows however a very satisfactory reproducibility, with respect to area as well as peak shape.

The above stability test was repeated with a crude lactate dehydrogenase extract, the same as that used in the large-scale purification experiment. The same apparatus and column were used as for the pure enzyme above, but the cycle time was extended from 12 min to 24 min. We could repeat the purification cycles 15 times (Fig. 7 shows cycles 1 and 15), before the performance and capacity of the column changed appreciably. If the column was dismantled and then repacked with the same fibre material, its capacity and general performance were restored, indicating that the change in performance was not due to any degeneration of the material itself.

CONCLUSION

The present work suggests that the affinity fibre material should be useful for separation and purification of enzymes and other macromolecules. The highest potential of the new bed material is probably in processing of large volumes with a low enzyme content, where the very high flow-rate possible with an affinity fibre bed is very desirable.

The very rapid adsorption possible with affinity fibres should also be very advantageous when isolating sensitive enzymes from crude extracts containing proteolytic activity.

The rapid equilibration between the non-porous fibre and the mobile phase, should also be beneficial for resolution, speed and general performance in analytical systems.

The advantages of non-porous fibre supports are obviously not limited to (bio)affinity systems. Ion-exchange and reversed-phase/hydrophobic interaction chromatography, are also good candidates for the application of the fibre concept. We are now investigating these areas.

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REFERENCES

1 W. B. Jakoby (Editor), *Methods in Enzymology, Vol. 104, Enzyme Purification and Related Techniques*, Academic Press, New York, 1984.

2 W. B. Jacoby and M. Wilchek (Editors), *Methods in Enzymology, Vol. 34, Affinity Techniques*, Academic Press, New York, 1974.
3 S. Ohlson, L. Hansson, P.-O. Larsson and K. Mosbach, *FEBS Lett.*, 93 (1978) 5.
4 P.-O. Larsson, M. Glad, L. Hansson, M. O. Månsson, S. Ohlson and K. Mosbach, *Adv. Chromatogr.*, 21 (1983) 41.
5 C. R. Lowe, M. Glad, P.-O. Larsson, S. Ohlson, D. A. Small, T. Atkinson and K. Mosbach, *J. Chromatogr.*, 215 (1981) 303.
6 D. J. Anderson and R. R. Walters, *J. Chromatogr.*, 376 (1986) 69.
7 K. Nilsson and P.-O. Larsson, *Anal. Biochem.*, 134 (1983) 60.
8 J. R. Sportsman, J. D. Liddil and G. S. Wilson, *Anal. Chem.*, 55 (1983) 771.
9 D. A. P. Small, T. Atkinson and C. R. Lowe, *J. Chromatogr.*, 266 (1983) 151.
10 M. Lindberg, P.-O. Larsson and K. Mosbach, *Eur. J. Biochem.*, 40 (1973) 187.
11 F. E. Regnier and R. Noel, *J. Chromatogr. Sci.*, 14 (1976) 316.
12 R. Axén, H. Drevin and J. Carlsson, *Acta Chem. Scand.*, B29 (1975) 471.
13 K. Nilsson and K. Mosbach, *Biochem. Biophys. Res. Commun.*, 102 (1981) 449.
14 M. Bradford, *Anal. Biochem.*, 72 (1976) 248.
15 B. G. Johansson, *Scand. J. Clin. Lab. Invest.*, 29, suppl. 124 (1972) 7.
16 B. Lunhd, *Clin. Chim. Acta*, 16 (1967) 305.
17 P. A. Bristow, P. N. Brittain, C. M. Riley and B. F. Williamson, *J. Chromatogr.*, 131 (1977) 57.
18 J. J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 129.
19 E. A. Hill and M. D. Hirtenstein, *Advances in Biotechnological Processes*, Vol. 1, A. R. Liss, New York, 1983, p. 31.
20 J. J. Holbrook, A. Liljas, S. J. Steindel and M. G. Rossmann, in P. D. Boyer (Editor), *The Enzymes*, Vol. XI, Academic Press, New York, 1975, p. 284.